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Gametophyte of the Andean Fern *Cheilanthes pilosa* Goldm. (Pteridaceae)

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ABSTRACT.—The gametophytic generation of *Cheilanthes pilosa* (Pteridaceae), including spore germination, morphological development of the gametophytes, major vegetative features and sexual expression, was studied. In *C. pilosa* spore germination was of the *Vittaria* model and the developmental pattern was intermediate between *Adiantum* and *Ceratopteris* models. Adult gametophytes were cordate and hairy, with unicellular hairs located in the margins and both the ventral and dorsal surfaces of the prothalli. Gametangia were of the normal type described for leptosporangiate ferns. In *C. pilosa* populations the gametophytes produced at first instance a high proportion of female gametophytes, few male gametophytes developed and most of the female gametophytes became bisexual with time. Thus, although outbreeding is possible, this species seemed to be promoting intragametophytic selfing as the major reproductive strategy.

KEY WORDS.—gametophyte, development, reproduction, Andes, Peru, *Cheilanthes pilosa*

Cheilanthes Sw. (Pteridaceae) is a large genus that comprises around 150–200 species. It is a cosmopolitan genus, but most species inhabit semi-arid locations of tropical and subtropical areas (Tryon, 1990). The genus presents three major biodiversity centers (Tryon and Tryon, 1973): one in America (from the USA-Mexico zone, where it is very abundant, until the Andes), another in Australia and the third in the Mediterranean basin, especially Greece and the Iberian Peninsula. It also appears in SE Africa. *Cheilanthes pilosa* Goldm. is a saxicolous species that occurs in the Andean areas of Peru and Bolivia.

The gametophytic phase of *Cheilanthes* has been subject of abundant literature. Among others, apogamy (Whittier, 1965; 1970), physiology and ecophysiology (Quirk and Chambers, 1981; Nondorf *et al.*, 2003; Palmieri and Swatzell, 2004) and morphology and development (Pray, 1961; Nayar, 1963; Pangua and Vega, 1996; Gabriel y Galán and Prada, 2009) have been studied in the genus. The aim of this work was to study spore germination, the prothallial development, the morphology of the adult gametophyte and the sexual behavior of *C. pilosa*.

MATERIAL AND METHODS

Spores for cultures were taken from sporophytes collected in Peru, Cuzco Department, Urubamba Province. The following is the location of the

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collection: Ollantaytambo, pasado Huilloq, 13°14′50.5″S 72°15′28.8″W, 2.920 m, roquedo, Gabriel y Galán s/n, 28/04/2008. The material was identified following Tryon and Stolze (1989). Vouchers are deposited in MA.

Each spore sample for cultures were taken from separate sporophytes kept dry at room temperature for around 8 months. Gametophytes were grown under fluorescent light on a 12-h light, 12-h dark cycle at 20 ± 2°C, in 6 cm plastic Petri dishes. Multispore cultures on mineral agar medium (Dyer, 1979) were established by sacking fertile pinnae on a weigh paper, and placing the obtained spores in the Petri dishes. The sowing was replicated twice for each sample. Percentage of germination was recorded on every third day for a random sample of 50 spores from each of the two plates, until the maximum germination was reached. Spore sizes given are the mean of 30 measurements. To study the stages of gametophyte development, random samples were taken weekly, from the beginning of spore germination until sexual maturity. Gametophytes were stained with chloral hydrate acetocarmine (Edwards and Miller, 1972), mounted in water and observed under a light microscope. Some in vivo observations were also made.

Results

The spores of *C. pilosa* were trilete, spherical, 39.2 μm in polar view, and presented the perispore with prominent cristae that tended to form a net of irregular areolae. Germination started about 5–7 days after sowing, when few spores (ca. 2%) showed a first rhizoid. Later, the first prothallial cell emerged, perpendicularly to that of rhizoid. After the first 7 days in culture, germination increased rapidly, reaching ca. 60% in the following 7 days and a maximum of 83% during the next week. Figure 1 shows the progress of the germination percentages over the days considered.

The development of the gametophyte of *C. pilosa* was very quick. Three to four days after germination, short filaments of 3–4 cells (ca. 150 μm in length) were formed (Fig. 2A). The apical cell underwent two oblique longitudinal divisions that produced 3 daughter apical cells. In one of them, a new division led to the formation of a first hair (Fig. 2B). Following transversal and longitudinal divisions in the apical and in the 2–3 subapical cells, formed a spatulate gametophyte of about 4–5 cells broad. The first 2–4 hairs developed in an alternate way in both the margins (Fig. 2C). Around 15 days after germination, these spatulate gametophytes organized an apical meristem by longitudinal divisions in the apex (Fig. 2D), which led to the development of a pre-cordate prothallus (Fig. 2E). At this stage, ca. 25–30 days after germination, the wings were beginning to develop, while the margins of the plate continued forming hairs (Fig. 2F), some of them as culmination of marginal projections. In ca. 40 days after germination, adult gametophytes were completely developed. These gametophytes were broad, of about 2 mm in width, hairy and symmetrically cordate (Fig. 2G).

The rhizoids of *C. pilosa* had the capacity of abruptly changing the direction of elongation, by causing the subapical region to grow in another direction

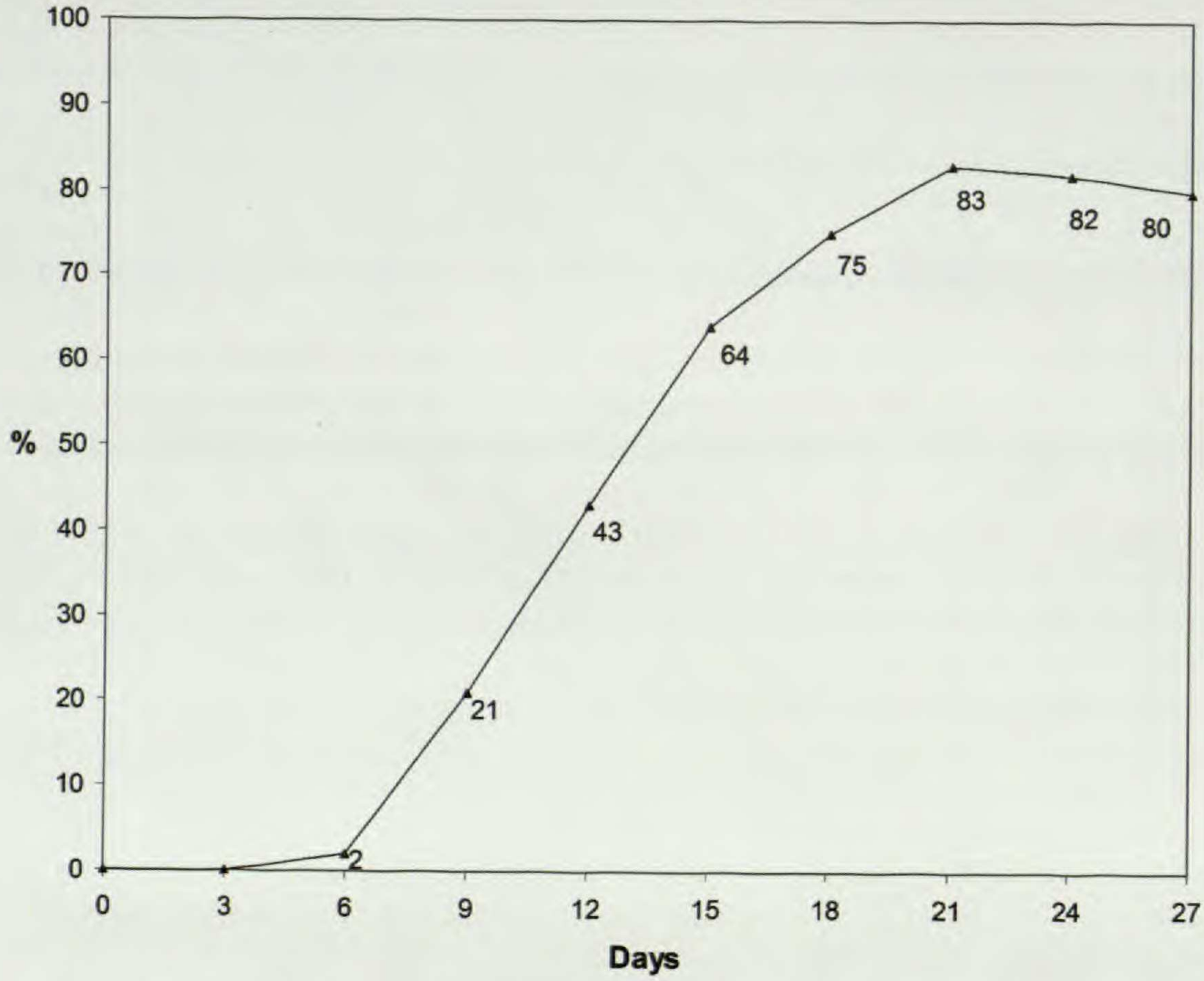

FIG. 1. Changes in the germination percentages of *Cheilanthes pilosa*, over 27 days.

from the apical region (Fig. 3A). In young and adult gametophytes of *C. pilosa*, numerous unicellular hairs could be seen (Figs. 2C–G), located in the margin and in both dorsal and ventral surfaces. The size of the hairs was ca. 50 μm. The marginal ones were subtended by a somewhat spindle-shape cell (Fig. 3B).

Cheilanthes pilosa developed gametangia 40 days after germination. Firstly, archegonia were relatively abundant, present immediately behind the apical notch. Female gametophytes were the only fertile ones in the cultures for a period of around 20 days, reaching ca. 60% of the prothalli; the rest were sterile. Later, some female gametophytes began to produce antheridia and became bisexual. Antheridia developed less frequently, and were located in the vicinity of the archegonia (Fig. 3C). For the rest of the observation period (ca. 250 days) bisexual prothalli became predominant in the cultures, reaching ca. 60%. Nevertheless, ca. 30% of the gametophytes were always female and ca. 10% were sterile. Strictly unisexual male gametophytes were observed occasionally, but unlike the bisexual ones, developed antheridia in great number (Fig. 3D).

DISCUSSION

The spores of *C. pilosa* show sizes and perispore ornamentations that fall in the normal ranges of variation and types previously reported for the genus

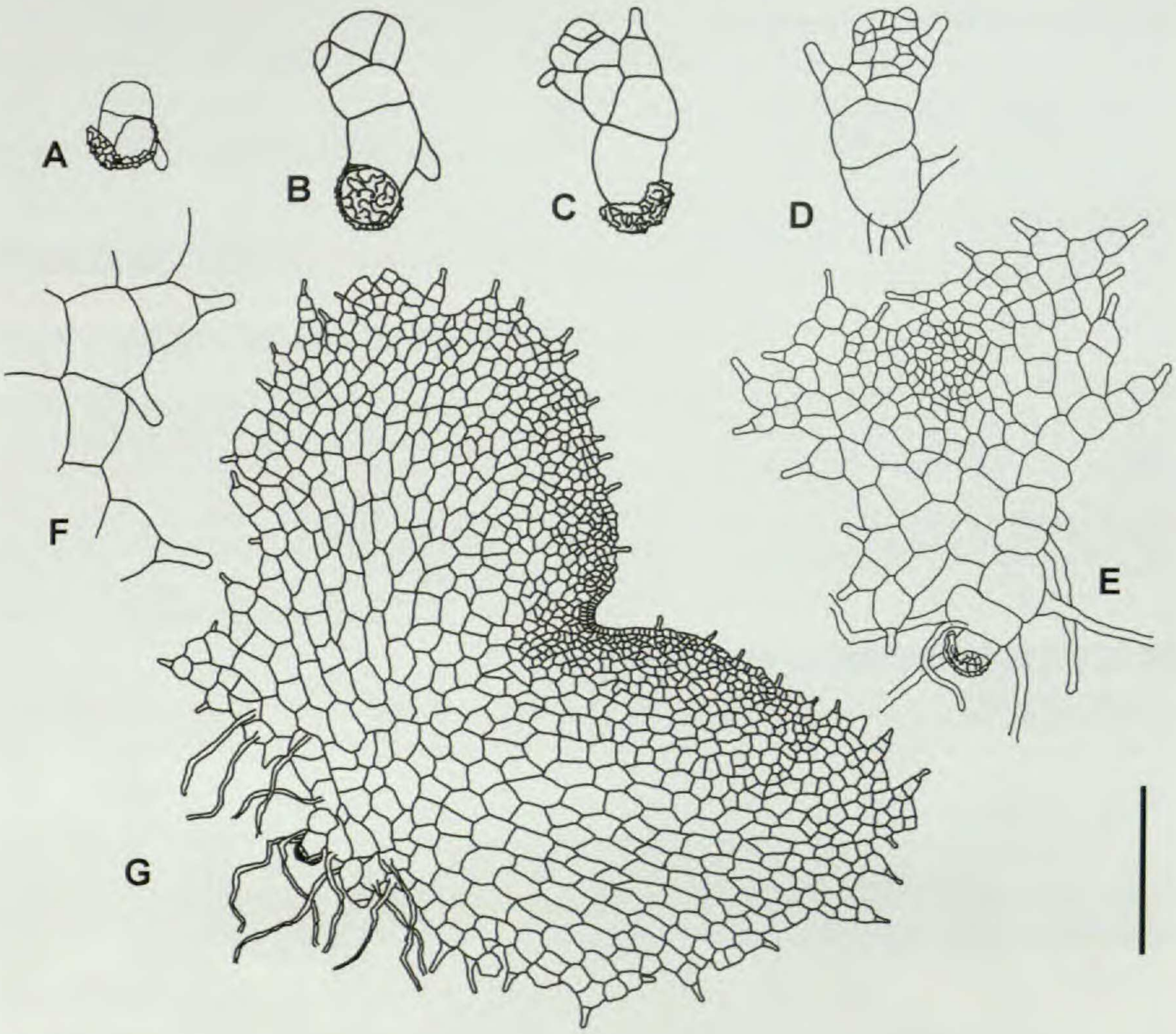

FIG. 2. Gametophyte development of *Cheilanthes pilosa*. A: Initial filament stage, 3 days. B: 3-celled filament with first longitudinal divisions in apical cell, 7 days. C: spatulate gametophyte with production of first hairs, 10 days. D: spatulate gametophyte with the beginning of the apical meristem, 15 days. E: initial cordate stage, 28 days. F: detail of marginal hairs, 28 days. G: adult prothallus, 40 days. Days are measured from the germination. Bar = 100 μm in F; 180 in A–D; 250 μm in E; 420 μm in G.

(Tryon and Lugardon, 1990). The spore germination is of the *Vittaria* model, in which the first rhizoid and the first prothallial cell emerged perpendicularly one to each other. This is the most common way of germination in the leptosporangiate ferns (Nayar and Kaur, 1968) and has been reported in other *Cheilanthes* species (Gabriel y Galán and Prada, 2009).

Gametophyte developmental process in *C. pilosa* is intermediate between the *Adiantum* model, in which an apical meristematic cell is formed in early stages, and the *Ceratopteris* model, in which the meristem is developed in a bidimensional plate from marginal cells (Nayar and Kaur, 1969). In *C. pilosa*, the meristem is formed in a spatulate bidimensional prothallus but from an apical cell. This kind of intermediate condition has been previously reported from other *Cheilanthes* species, as *C. aemula* Maxon, *C. leucopoda* Link and *C. meifolia* Eaton (Nayar and Kaur 1969), *C. tinaei* Tod. and *C. acrostica* (Balb.)

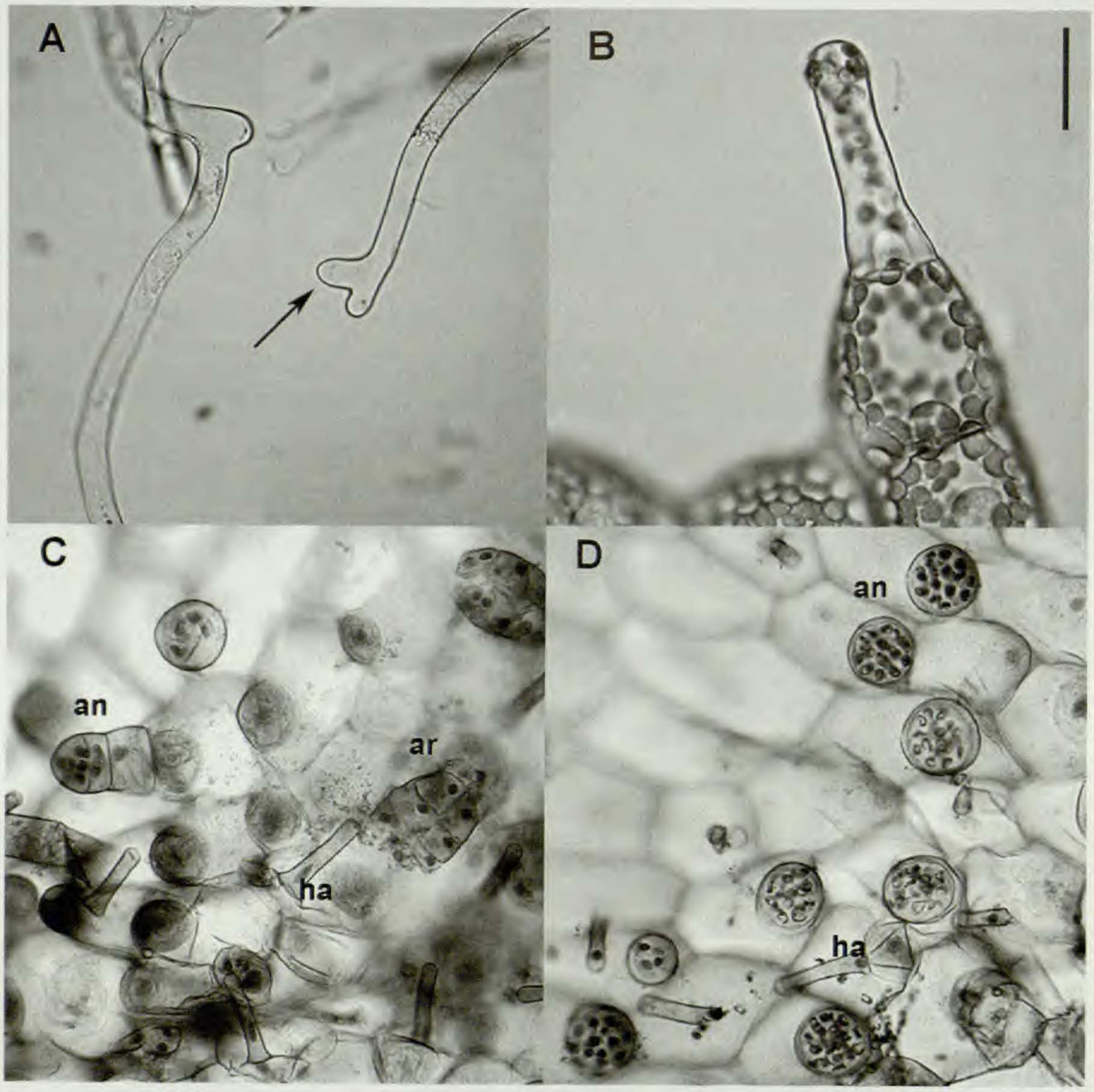

FIG. 3. Vegetative and reproductive features of *Cheilanthes pilosa*. A: rhizoids, showing subapical elongation (arrow), on a young cordate prothallus, 30 days. B: marginal hair, subtended by a spindle-shape cell, on an adult sexual prothallus, 50 days. C: gametangia on bisexual gametophyte, 80 days. D: antheridia on male gametophyte, 70 days. an=antheridium; ar=archegonium; ha=hair. Days are measured from the germination. Bar = 35 μm in A; 20 μm in B, C; 15 μm in D.

Tod. (Pangua and Vega 1996), and *C. glauca* (Cav.) Mett. (Gabriel y Galán and Prada, 2009). It seems to be the normal way of gametophyte development in the genus, while the typical *Adiantum* and *Ceratopteris* models are uncommon (Nayar & Kaur 1971). In addition, unlike most of the above mentioned species, *C. pilosa* formed marginal hairs from early stages of the development process.

In general, *Cheilanthes* is known for producing hairless gametophytes. Atkinson (1973) stated that, among the Pteridaceae, the majority of genera present hairless gametophytes, and those with hairy gametophyte are confined to the *Notholaena* group. Nayar and Kaur (1971) said that all genera in the Cheilanthaceae are naked, except for *Notholaena*, although some of the

examples they used are now considered under *Cheilanthes* (*N. galeottii* Fée, accepted name *Cheilanthes galeottii* (Fée) Mickel & Beitel). We also know of the naked gametophytes of *C. alabamensis* (Buckley) Kunze (Whittier, 1965), *C. tinaei* and *C. acrostica* (Pangua and Vega, 1996) and *C. glauca* (Gabriel y Galán and Prada, 2009). In addition, there are gametophytes of *Cheilanthes* (Giauque, 1949; Nayar and Kaur, 1971) and *Notholaena* (Tryon, 1947; Nayar and Kaur, 1971; Atkinson, 1973) that are known to be glandular, even producing wax-secretions. Currently, the presence of wax-farinose glands on gametophytes is considered to be a synapomorphy of *Notholaena* (Rothfels *et al.*, 2008). In conclusion, *Cheilanthes* presents naked gametophytes as the rule but also rarely hairy gametophytes, like *C. galeottii* (hairs 2-celled) and *C. pilosa* (hairs unicellular). A reexamination of the information on the gametophytes of the group is necessary to understand if gametophyte characters can be used to support the modern revisions of the family (Gastony and Rollo, 1995; Schuettpelz *et al.*, 2007).

The sex organs in *C. pilosa* were of the normal type defined for the leptosporangiate ferns (Nayar and Kaur, 1971). Although it has been said that the normal sequence of gametangia development in the lepotsporangiate ferns begins with the antheridia and continues with the archegonia (Atkinson and Stokey, 1964; Nayar and Kaur, 1971), there are many deviations reported. That is the case of *C. pilosa*. The great initial production of female gametophytes in *C. pilosa* coincides with other species, as *C. tinaei* (Pangua and Vega, 1996), but differs from others, as *C. acrosticha* (Pangua and Vega, 1996) and *C. glauca* (Gabriel y Galán and Prada, 2009), in which the sexual expression begins with a great proportion of male gametophytes. The scarce number of male gametophytes in the populations of *C. pilosa* is indicative of a low rate of intergametophytic unions, while the increasing development of bisexual prothalli from the females promotes the intragametophytic reproduction. The occurrence of this last strategy is also supported by the very close location of both types of gametangia.

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